Available online at www.sciencedirect.com
ScienceDirect
Procedia Engineering 5 (2010) 41–44
Procedia Engineering
www.elsevier.com/locate/procedia

Proc. Eurosensors XXIV, September 5-8, 2010, Linz, Austria

Continuous separation of viable cells by travelling wave dielectrophoresis

Sander van den Driesche[a,*], Vivek Rao[a], Dietmar Puchberger-Enengl[a], Wojciech Witarski[b], Michael J. Vellekoop[a]

[a]*Institute of Sensor and Actuator Systems, Vienna University of Technology, Gusshausstrasse 27-29, 1040, Vienna, Austria*
[b]*Institute of Virology, Slovak Academy of Sciences, Dubravska Cesta 9, 84245, Bratislava, Slovak Republic*

Abstract

In this contribution we present a label-free continuous separation device for suspended-grown biological cells based on travelling wave dielectrophoresis (twDEP). Suspended-grown cells could otherwise only be separated by flow-cytometry which requires elaborate labelling steps. The biochip consists of a structured PDMS layer on glass in which the channels have been defined. Parallel electrodes positioned along the microfluidic channel were used to expose selected cells to a twDEP force perpendicular to a pressure driven flow. With this device we have successfully separated suspended-grown viable Jurkat (T-cell leukaemia) cells from a mix also containing non-viable cells and *L. casei* bacteria.

Keywords: Cell separation, travelling wave dielectrophoresis, bacteria contamination

1. Introduction

Viable biological cells growing attached to a surface can easily be separated from cell culture debris and bacterial contamination by changing the culture medium. For suspended growing cells however, this is not an option. One way to separate suspended viable cells is by using flow cytometry, requiring expensive elaborate labelling steps. A phenomenon that does not require labelling for separation is dielectrophoresis, defined as a motion of polarizable particles exposed to a non-uniform electric field. Many dielectrophoretic separation devices require accurate hydrodynamic focusing [1] or multiple frequencies [2, 3] to position the sample into the separation section of the microchannel. The use of 3D electrode geometries have been shown which require highly accurate alignment steps during the fabrication process [4]. Also a non-continuous sample separation device based on twDEP has been shown where the electrodes were positioned perpendicular in the microfluidic channel [5]. In previous work we have shown a continuous separation based on positive and negative DEP without the need of accurate sample prefocusing. Viable Jurkat cells experiencing pDEP were separated from a mix containing also cell debris and non-viable cells by being guided towards and dragged along the edge of a planar electrode [6]. In this contribution we still use planar electrodes but now positioned over the whole width of the microfluidic channel allowing higher electric field strengths (Fig. 1).

Instead of the in-phase component of the dipole moment (real part of the Clausius-Mossotti factor, f_{cm}) we used the imaginary part of f_{cm}, the out-of-phase component, allowing twDEP. This separation method is based on the difference

*Corresponding author. Tel.:+43-1-58801-36670; fax: +43-1-58801-36699.
Email address: sander.driesche@tuwien.ac.at (Sander van den Driesche)

1877-7058
doi:10.1016/j.proeng.2010.09.043

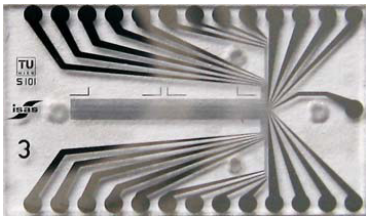

Figure 1: Photograph of the 19 x 11 mm^2 travelling wave DEP separation device. The chip contains 27 parallel electrodes, 20 μm in width and 1 cm in length with a gap size of 20 μm, each phase-shifted 90° to obtain a travelling electric field. The microfluidic channel was 30 μm in height.

in relaxation times between particle and suspending medium in a phase-shifted AC electric field. A positive out-of-phase component indicates that the dipole lags behind the electric field. A positive $\Im(f_{CM})$ results in a net force along the co-field, a negative $\Im(f_{CM})$ to particle movement away from the co-field.

$$f_{cm} = \frac{{\varepsilon_p}^* - {\varepsilon_m}^*}{{\varepsilon_p}^* + 2{\varepsilon_m}^*} \quad \text{, with} \quad \varepsilon^* = \varepsilon - j\left(\frac{\sigma}{\omega}\right) \tag{1}$$

From Eq. 1 the imaginary (out of phase) part of the Clausius-Mossotti factor can be derived:

$$\Im[f_{cm}] = \frac{(\varepsilon_p - \varepsilon_m)\left(\frac{\sigma_p+2\sigma_m}{\omega}\right) - (\varepsilon_p + 2\varepsilon_m)\left(\frac{\sigma_p-\sigma_m}{\omega}\right)}{(\varepsilon_p + 2\varepsilon_m)^2 + \left(\frac{\sigma_p+2\sigma_m}{\omega}\right)^2} \tag{2}$$

The twDEP force equation for first order induced dipoles reads as follows:

$$F_{twDEP} = -\frac{4\pi^2 r^3 \varepsilon_m}{\lambda} \Im\left|f_{cm}(\omega)\right| E_{rms}^2 \quad \text{, with} \quad \Im\left|f_{cm}\right| \in [-0.75...0.75] \tag{3}$$

where ε is the permittivity and σ the conductivity of the surrounding medium m or suspended particle p, r the particle radius, ω the angular frequency, E the electric field strength and λ the wavelength of the travelling field (the distance between four electrodes when a 0°, 90°, 180°, and 270°phase shift is used).

2. Material and Methods

2.1. Sample Preparation

Jurkat cells (an acute T-cell leukemia cell line) were cultivated in culture flasks at 37 °C and 5% CO_2. As growth medium, Dulbecco's modified eagle medium containing 4.5 g/L glucose, 10% fetal calf serum, 2 mM L-glutamine, and antibiotics(100 units/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml Amphotericin B) was used (all obtained from Lonza Bioscience). The *L. casei* bacteria (DSM 20011) were washed in PBS and added to the sample mix. The strong influence of the culture medium conductivity on the DEP phenomena was prevented by washing the cell mix twice in 5 mL low conductivity DEP medium. This medium consisted of 10% w/w sucrose and 2% w/w dextrose to compensate for the osmotic pressure on the cells. By adding phosphate-buffered-saline (pH 7.2) the final DEP medium conductivity was set to 40 mS/m (measured with HANNA HI9835).

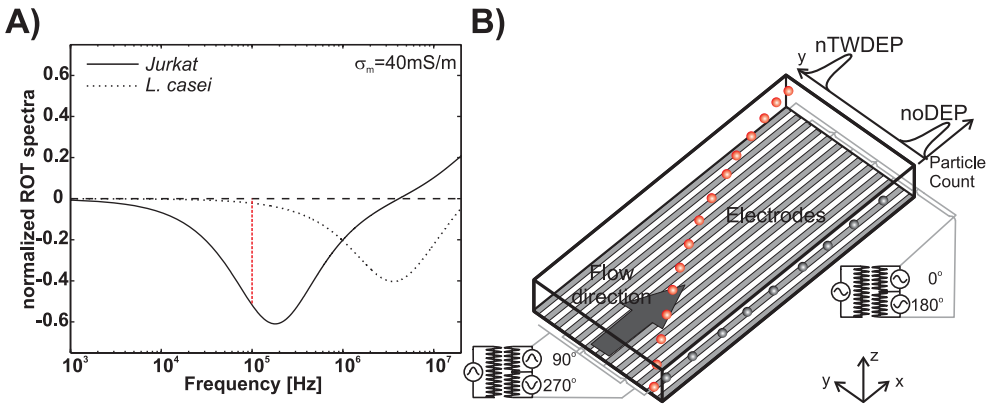

Figure 2: **A)** ROT spectra of Jurkat cells and *L. casei* bacteria resuspended in a low conductivity buffer, ε_m = 40 mS/m. At a travelling wave electric field frequency of 100 kHz Jurkat cells experience a force away from the co-field while *L. casei* bacteria are hardly influenced. **B)** Separation based on travelling wave dielectrophoresis. The sample is released at the right side of the channel and is dragged through the channel by a pressure driven flow. Selected cells exposed to a non-uniform phase-shifted electric field experiencing twDEP move away from the co-field (0°- 270°- 180°- 90°direction) leaving the channel at the opposite channel wall.

Figure 3: Separation of Jurkat cells by a travelling electric AC field. **A)** Cells enter the device on one side of the channel. **B)** The targeted cells are moving against the applied co-field towards the other side of the channel. **C)** Separated cells leaving the channel.

2.2. Cell Characterization

Separation of biological samples by means of twDEP is based on differences in cell size and rotational velocity (ROT) spectra. Before separating suspended growing viable Jurkat cells from a mix, also containing cell culture debris and *L. casei* bacteria, the cells and bacteria have been characterized by being exposed to a rotating electric field. The cell rotations per time unit were extracted from captured video frames. Only cells located near the centre of the electrodes and at least four cell diameters away from each other were used to avoid the influence of a dipolar field of neighbouring cells [7]. Besides in size and shape (spherical 12 µm diameter versus rod shaped 1 x 2 μm^2) there is also a clear difference in their ROT spectra (Fig. 2A).

3. Results and Discussion

Before introducing the bacteria contaminated biological mix into the separation device the mix was suspended in 40 mS/m DEP medium. The viable Jurkat cells were experiencing a perpendicular twDEP force away from the co-field when being dragged through the channel by a pressure driven flow. In Fig. 2B the separation principle is depicted. In Fig. 3 three frames are depicted showing the successful separation of twDEP experiencing Jurkat cells. In the first frame the cells are introduced into the separation channel, the second frame shows targeted cells moving towards the other side of the channel away from the co-field, and in the last frame the cells are leaving in the opposite side of the channel from which they were introduced.

The cell mix was introduced at one side of the channel and focussed to the 0 to 70 µm y-region by a sheath flow applied from the opposite side. In Fig. 4 the bars of the DEP off histogram represents the y-coordinates of the Jurkat cells leaving the separation channel without an applied electric field. After switching on the DEP voltage (3.5 V_{RMS},

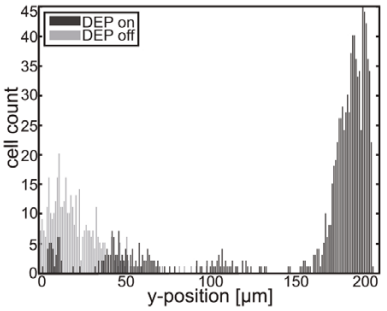

Figure 4: Viable Jurkat cells separated from cell culture debris containing non-viable cells and *L. casei* bacteria. The sample stream (0.1 µL/min) was introduced in the y-region of 0 to 70 µm by a sheath flow of 0.15 µL/min (DEP off bars). By applying a phase-shifted voltage of 3.5 V at 100 kHz a travelling electric field was obtained. The selected viable Jurkat cells moved away from the co-field (in 0°-270°-180°-90°direction) towards the other side of the channel (y-region of 170 to 200 µm). *L. casei* bacteria were not considered in the cell count because of the incompatibility with our particle recognition software due to their small size, but left the separation channel in the y-region between 0 and 50 µm (seen under a microscope).

f = 100 kHz) a clear separation of viable Jurkat cells was observed. These selected cells moved away from the co-field towards the (y-region of 170 to 200 µm) of the channel. *L. casei* bacteria are due to their much smaller volume and different electrical parameters (see Eq. 2 and Fig. 2) not affected under the used separation conditions and left the separation channel in the 0 to 70 µm y-region (the bacteria were not considered in the cell count because of the incompatibility with our particle recognition software).

4. Conclusions

With parallel twDEP electrodes positioned along the microfluidic channel we are able to deflect cells of interest towards the other side of the channel. Our experiments show a nearly perfect separation of suspended-grown viable Jurkat cells from a bacteria contaminated cell mix which could otherwise only be separated by flow-cytometry requiring elaborate labelling steps.

Acknowledgments

This project is a part of an EU Marie Curie Research Training Network (MRTN): On-Chip Cell Handling and Analysis, CellCheck. Proj.no.MRTN-CT-2006-035854. The authors would like to acknowledge A. Jachimowicz, E. Svasek and P. Svasek from the Sensor Technology Lab at ISAS and the Center for Micro- and Nanostructures (ZMNS), TU Vienna. The *L. casei* bacteria were kindly provided by H. Wiesinger-Mayr, AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria.

References

[1] Y. Li, C. Dalton, H. J. Crabtree, G. Nilsson, K. V. I. S. Kaler, Continuous dielectrophoretic cell separation microfluidic device, Lab Chip 7 (2) (2007) 239–248. doi:10.1039/b613344d.

[2] T. Braschler, N. Demierre, E. Nascimento, T. Silva, A. G. Oliva, P. Renaud, Continuous separation of cells by balanced dielectrophoretic forces at multiple frequencies, Lab Chip 8 (2008) 280–286. doi:10.1039/b710303d.

[3] L. Wang, J. Lu, S. A. Marchenko, E. S. Monuki, L. A. Flanagan, A. P. Lee, Dual frequency dielectrophoresis with interdigitated sidewall electrodes for microfluidic flow-through separation of beads and cells, Electrophoresis 30 (2009) 1–10. doi:10.1002/elps.200800637.

[4] I.-F. Cheng, V. E. Froude, Y. Zhu, H.-C. Chang, H.-C. Chang, A continuous high-throughput bioparticle sorter based on 3d traveling-wave dielectrophoresis., Lab Chip 9 (22) (2009) 3193–3201. doi:10.1039/b910587e.

[5] L. Cui, H. Morgan, Design and fabrication of travelling wave dielectrophoresis structures, J. Micromech. Microeng. 10 (2000) 72–79. doi:10.1088/0960-1317/10/1/310.

[6] S. Kostner, S. van den Driesche, W. Witarski, S. Pastorekova, M. J. Vellekoop, Guided dielectrophoresis: a robust method for continuous particle and cell separation, IEEE Sens Jdoi:10.1109/JSEN.2010.2044787.

[7] P. Gascoyne, F. F. Becker, X. B. Wang, Numerical analysis of the influence of experimental conditions on the accuracy of dielectric parameters derived from electrorotation measurements, Bioelectrochemistry and Bioenergetics 36 (1995) 115–125. doi:10.1016/0302-4598(94)05015-M.